

## MORPHOLOGICAL AND TAXONOMICAL ASSESSMENT OF FORMER AND PRESENT RECORDS OF *PACHYCLADELLA UMBRINA* AND *P. ZATORIENSIS* (GREEN ALGA, CHLOROCOCCALES)

Olivier L. REYMOND<sup>(1)</sup>, Takaaki YAMAGISHI<sup>(2)</sup> and Jean-Claude DRUART<sup>(3)</sup>

(1) Conservation de la faune, Laboratoire d'hydrobiologie. Ch. du Marquisat 1, CH-1025 St-Sulpice, Switzerland.

(2) Nihon University, College of agriculture and veterinary medicine, 1866, Kameino, Fujisawa-shi, Kanagawa-ken, 252 Japan.

(3) Institut de Limnologie, Station d'Hydrobiologie lacustre (INRA), 75 av. de Corzent, B.P. 511, F-74203 Thonon Cedex, France.

**ABSTRACT** - The original description of the genus *Pachycladella* (G.M. Smith) Silva and its type-species *P. umbrina* (G.M. Smith) Silva by G.M. Smith (1924) mentions explicitly that the four bifurcated appendages which decorate this unicell globose alga show a cruciate position and very rarely a tetrahedral one. However a compiling of subsequent reports shows that this morphological feature has been often passed over or modified without any expressed reasons. It results in a biased view of the genus as well as a confusion about the real morphology of *Pachycladella umbrina* and *P. zatoriensis* (Bednarz et Mrozinska-Webb) Komárek.

Former reports and new morphological and morphometrical records of both species show that cells with cruciate appendages are *P. umbrina*, and cells with tetrahedrally positioned appendages are *P. zatoriensis*. Based on the present LM (light microscopy) and former TEM (transmission electron microscopy) results, slightly changed descriptions are given of *Pachycladella* and *P. umbrina*. Finally, short comments are given on all known taxa of *Pachycladella*, making this report a general survey of the genus.

**RÉSUMÉ** - La diagnose du genre *Pachycladella* (G.M. Smith) Silva et de son espèce-type *P. umbrina* (G.M. Smith) Silva par G.M. Smith (1924) mentionne explicitement que les quatre appendices bifurqués qui décorent cette algue unicellulaire coccoïde, ont une disposition en croix, très rarement une disposition tétraédrique. Cependant la compilation de différents travaux, montrent que cette particularité morphologique a été souvent occultée ou modifiée sans raisons apparentes. Il en résulte une vision peu claire du genre ainsi qu'une confusion sur la morphologie exacte de *Pachycladella umbrina* et *P. zatoriensis* (Bednarz et Mrozinska-Webb) Komárek.

Des publications antérieures ainsi que la présente étude morphologique et morphométrique montrent que les deux espèces peuvent se distinguer par la disposition cruciée (*P. umbrina*) ou tétraédrique (*P. zatoriensis*) de leurs appendices. En se basant sur des études en microscopie électronique ainsi que sur les présents résultats, les auteurs proposent une description légèrement modifiée du genre *Pachycladella* et de *P. umbrina*. En dernier lieu, de brefs commentaires sur tous les taxa du genre *Pachycladella* complètent ce rapport.

**KEY WORDS :** Algae, Chlorophyceae, Chlorococcales, *Pachycladella*, morphology, taxonomy.

The genus *Pachycladella* (G.M. Smith) Silva (1970) concerns planktonic, coccoid, and very rare Green algae characterized by a globose cell-body surrounded by four or more tubular appendages with a blunt or a bifurcated apex.

A morphological detail in the original description of the genus and its type-species *P. umbrina* by G.M. Smith (1924) initiated the present report: "...Cell wall bearing four quadrately (very rarely pyramidately) arranged appendages...". An analyze of the literature made us discover that this morphological feature has very often been passed over or modified. Moreover, numerous observations of field material helped us to establish that the characteristic described by Smith was specific to *P. umbrina* and not generic.

One consequence of the alteration to Smith's description was a confusion between *P. umbrina* with quadrate (also called cruciate) appendages and another species which was only very recently described as the new taxon *P. zatoriensis* (Bednarz et Mrozinska-Webb, 1971) Komárek (1979).

This report will first present a critical and historical survey of the main papers dealing with *Pachycladella*. It will be followed by the description and the assessment of several original records of *P. umbrina* and *P. zatoriensis*. These will explain why the characteristic mentioned above (quadrately arranged appendages) is in fact specific to the type-species *P. umbrina* and not generic. A modified description of the genus and the type-species will be given as a result of former TEM studies (Reymond & Hegewald, 1990; Reymond *et al.*, submitted) and the present LM report. Another goal of this report is to make a short survey of all the taxa which are included in the genus.

## A CRITICAL REVIEW

### *Bernardia tetraëdrica* Playfair (1918) or the first record of *Pachycladella*.

The first record of a *Pachycladella* species is certainly due to Playfair (1918) near Lismore, Australia (Fig. 1). His description is very accurate: "This species consists of a single spherical cell with (apparently) the membrane drawn out into four long very slender hollow spines, arranged tetraëdrically. The spines are blunt at the extreme tip, not acutely pointed, and the base are greatly inflated..." He calls this species *Bernardia tetraëdrica*, but in fact he probably describes what we determine now as *P. zatoriensis*. The drawing of Playfair has some similarities with *Treubaria* Bernard (1908), and Reymond (1980) made it erroneously a synonym of *T. triappendiculata*.

### *Pachycladon umbrinus* G.M. Smith (1924) or the second record of *Pachycladella*.

The second record of *Pachycladella* is due to G.M. Smith (1924) in the USA. The generic and specific descriptions are accompanied with drawings of the single type-species *Pachycladon umbrinus* (Fig. 2). Later Silva (1970) discovers that the name *Pachycladon* was already used for a Cruciferae flower and consequently proposes the name *Pachycladella umbrina*.

The description of the genus by G.M. Smith (1924) is very clear: "... Cell bearing four quadrately (very rarely pyramidately) arranged appendages". This means that all the cells belonging to this genus have appendages with a cruciate

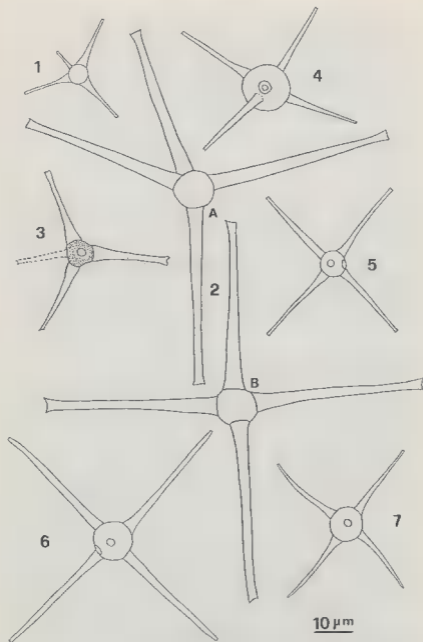


Fig. 1. *Bernardia tetraedrica*, redrawn from Playfair (1918). Fig. 2. *Pachycladon umbrinus*, redrawn from G.M. Smith (1924). Fig. 3. *Pachycladon zatoriensis*, redrawn from Bednarz & Mrozinska-Webb (1971). Fig. 4-7. *Pachycladella umbrina* from Taiwan.

disposition, and very rarely a tetrahedral one. We will see below that this feature is in fact not generic but specific: only *Pachycladella umbrina* has appendages with a cruciate disposition, whereas *P. zatoriensis* which is described much later, has appendages with a tetrahedral one (Fig. 3).

Smith (1924) mentions that the appendages are 35-50  $\mu\text{m}$  long. However he never mentions the number of cells observed and the length average of the processes. Consequently the words "very rarely pyramidately" are vague and unfortunately without any statistical base. In later descriptions, Smith (1933, 1950) does not mention new personal record, but uses the word "sometimes" instead of "very rarely".

#### The later reports.

Unfortunately the compilation of papers dealing with *Pachycladella* shows that the true description of the appendages dispositions in the diagnosis of the genus by G.M. Smith (1924) is later simply and incomprehensively passed over or modified. Several authors (i.e., Bourrelly, 1966, 1972; Fott & Kovacik, 1975; Komárek & Fott, 1983; Dillard, 1989) assert that the genus or its species *P. umbrina* is characterized by a tetrahedral disposition of the appendages, without any mention of the cruciate one. This induces an imprecision about the real morphology of *P. umbrina*. As a result García de Iñifiani *et al.* (1976) describe *P. umbrina* with four appendages in a tetrahedral disposition, and draw on the contrary, the picture of a cell with a cruciate disposition! In one case, we can assume that cells determined as *Pachycladella umbrina* are without any doubt *P. zatoriensis* (i.e., Capdevielle, 1985).

The report of Korshikov (1953, 1987) on *P. umbrina*, is an intermediate case where the original description by G.M. Smith is only slightly arranged: "...appendages arise arranged in the form of a cross or pyramidally...".

Prescott (1964) seems to be prudent but however distorts the original description; he uses the words "arm-like appendages radiating" without any mention of the spatial distribution. His drawing "redrawn from Smith" shows an original compromise of cruciate and tetrahedrally disposed conical processes with hypertrophied bifurcated apices.

On the contrary, Printz (1927) interprets and translates the description of G.M. Smith (1924) with accuracy "... 4 in einer Ebene quadratisch (nur selten tetraedrisch) angeordneten Stacheln ...". He cites the work of Playfair (1818) (see above) on *Bernardia tetraëdrica* and thinks that both species could be related: "... nähere Verwandtschaft ...". He is well advised and does not write "identische" (identical), but "nähere" (close). We can also note the excellent description of *P. umbrina* by Philipose (1967).

The record of *P. umbrina* by Wawrik (1977) is very well described. However it raises some questions: by the length of the appendages, the material is close to *P. umbrina* as described by G.M. Smith (1924), but because of the tetrahedral disposition of the same appendages this material could also be identified as a variety of *P. zatoriensis*, or eventually as a new species. It is interesting to note that one drawing (p. 61, fig. c) shows a cruciate disposition but all the others show a tetrahedral one. No numerical data are given concerning the cell number and their features.

In 1971, when Bednarz & Mrozinska-Webb describe *Pachycladon zatoriensis* (Fig. 3), they note that the main difference with *P. umbrina* is the length of the appendages. They do not pay particular attention to the spatial orien-

tation of the appendages, the tetrahedral disposition is not specifically mentioned. Fortunately, the accurate figures show this obvious characteristic (see also Bednarz & Novak, 1972).

In their report dealing with cultured *P. umbrina*, Raymond & Hegewald (1990) are not yet fully aware of this morphological problem. However they note: "...appendages placed approximately in the same plane and displaying a cruciate configuration. A tetraedric configuration is seen less frequently". Figures of their report confirm the text.

In other reports authors mention *P. umbrina*, but without any data allowing a determination at the present time (i.e., Whitford, 1936, 1943, 1958; McInteer, 1939; Lackey, 1958; Dillard *et al.*, 1976; Harmon Engin. & Test Comp., 1983; Metee *et al.*, 1984).

A chronological list of the previously cited as well as additional authors, the type of reports and a short description of all the taxa involved with *Pachycladella* is given in Table I.

## PRESENT RECORDS OF PACHYCLADELLA

### MATERIALS AND METHODS

#### Origin of the material

Four samples of phytoplankton containing *Pachycladella* cells have been examined by light microscopy.

The first sample (Yamagishi, No. 493) has been collected in May 1988 in an irrigation pond at Taoyuan (Taiwan) and preserved with formaldehyde. We will see below that all its *Pachycladella* cells were determined as *P. umbrina*. We must also call attention to the fact that, amongst the several species of *Mischococcales* and *Chlorococcales* of this sample, we find the very rare alga *Desmatraetum delicatissimum* Korschikov.

The three other samples have been collected in August 1990 in three reservoirs: La Noue, Le Haut Rançon, and La Velle, near Lyon (France). Samples were preserved in Lugol. Floristic data concerning these reservoirs have been recorded by J.-C. Druart in a report for "La Compagnie Générale des Eaux" at Montceau les Mines (France). We will see below that all the *Pachycladella* of these samples were determined as *P. zatoriensis*.

#### Light microscopy.

A drop of fixed phytoplankton was added to a drop of melted glycerin-gelatin on a microscope slide and protected with a coverslip. Observations were made with a 40x (dry) or a 50x (oil immersion) bright-field or phase-contrast objective. Every cell of *Pachycladella* was outlined with the help of a drawing tube, and the position and the length of the appendages as well the cell diameter were recorded. All the measurements are given in Table II.

The appendages are tubular or slightly conical structures of about 2-4  $\mu\text{m}$  in diameter at their bases. Despite these variations, the measurements of the diameters were not made systematically and the efforts were focused on the other features mentioned above.

YEAR	AUTHORS	TYPE OF REPORT	CITED TAXA	NF	NOF	CRU	TEF	APPEND. L.	CELL DIAM.	COMMENTS
1910	Playfair	Floris. System.	tetradedrica	1	1	-	+	12		zatoriensis?
1924	Smith	Ecol. System.	umbrinus	3	3	+	VR	35-50	8.5-12.5	umbrinus
1927	Frantz	Systematic	umbrinus	2	0	+	VR	*	*	umbrinus
1927	Frantz	Systematic	tetradedrica	0	0	+	*	*	*	zatoriensis
1931	Smith	Floris. System.	umbrinus	3	0	+	SO	35-50	8.5-12.5	umbrinus
1936	Whitford	Floris. Morphol.	umbrinus	0	0	-	*	35-50	8.5-15.5	prob. umbrinus
1939	Melabeer	Floristic	umbrinus	0	0	+	*	*	*	uncertain
1941	Whitford	Floristic	umbrinus	0	0	-	*	*	*	prob. umbrinus
1950	Smith	Floris. System.	umbrinus	2	0	+	SO	*	*	umbrinus
1951	Korshikov	Systematic	umbrinus	3	0	+	*	25-50	8.5-12.5	umbrinus
1958	Whitford	Ecol. Floris.	umbrinus	0	0	-	*	*	*	prob. umbrinus
1958	Lackey	Ecol. Floris.	umbrinus	0	0	+	*	*	*	uncertain
1960	Fott	Systematic	Pachycladon	0	0	+	*	*	*	NC
1964	Prescott	Identification	umbrinus	1	0	+	+	*	*	unclear
1965	Chadybowa & Chu.	Systematic	minus	6	6	+	+	5-9.3(>14)	3-5	uncertain
1965	Chadybowa & Chu.	Systematic	umbrinus	0	0	-	*	*	*	NC
1966	Bourrelly	Systematic	umbrinus	2	0	-	*	*	*	NC
1967	Phillipose	Systematic	umbrinus	1	1	+	SO	35-52.5	7.5-12.5	umbrinus
1970	Silva	Nomenclature	umbrinus	0	0	+	*	*	*	NC
1970	Silva	Nomenclature	minor	0	0	+	*	*	*	NC
1971	Bodnarz & Nroz.	Systematic	zatoriensis	2	2	-	+	17-20	6-10	zatoriensis
1972	Bodnarz & Nowak	Floristic	Pachycladon	1	1	-	+	17.2-19.8	6.7-9.9	zatoriensis
1972	Bourrelly	Systematic	umbrinus	2	0	-	+	*	*	NC
1973	Whitford & Schu.	Systematic	umbrinus	1	0	-	+	*	*	NC
1975	Fott & Kováčik	Systematic	umbrinus	1	0	-	+	17-50	6-10	NC
1975	Fott & Kováčik	Systematic	minus	0	0	+	*	*	*	NC
1975	Fott & Kováčik	Systematic	zatoriensis	0	0	+	*	17-20	*	NC
1975	Fott & Kováčik	Systematic	konarekii	4	4	+	+	18-20	7-10	uncertain
1976	Barta & al.	Identification	umbrinus	2	0	+	-	35-50	8.5-12.5	NC
1976	Dillard & al.	Floristic	umbrinus	0	0	+	*	*	*	uncertain
1976	Garcia Kn. & al.	Floris. System.	umbrinus	1	1	+	+	15	5	umbrinus
1977	Wawrik	Floris. System.	umbrinus	7	7	+	+	27-50	6.6-11	uncertain
1977	Wawrik	Floris. System.	minus	*	*	+	*	*	*	NC
1979	Konárek	Systematic	zatoriensis	0	0	+	*	*	*	NC
1979	Reymond	Systematic	tetradedrica	0	0	+	*	*	*	NC
1980	Reymond	Systematic	umbrinus	0	0	+	*	*	*	NC
1980	Reymond	Systematic	zatoriensis	0	0	+	*	*	*	NC
1980	Reymond	Systematic	konarekii	0	0	+	*	*	*	NC
1983	Barmon E. & T.C.	Ecology	umbrinus	0	0	+	*	*	*	uncertain
1983	Jha & Kausbal	Floristic	gobindsagar.	0	0	+	*	96-120	10-12.8	umbrinus
1983	Konárek & Fott	Systematic	umbrinus	1	0	+	+	27-50	6-12.5	NC
1983	Konárek & Fott	Systematic	minor	4	0	+	+	5-8.5(>14)	3-5	NC
1983	Konárek & Fott	Systematic	zatoriensis	2	0	-	+	17-20	6-10	NC
1983	Konárek & Fott	Systematic	konarekii	3	0	-	+	18-20	7-10	NC
1984	Metee & al.	Ecology	Pachycladon	0	0	+	*	*	*	uncertain
1984	Reymond & Koum.	Systematic	umbrinus	0	0	+	*	*	*	NC
1985	Cepdevielle	Floristic	umbrinus	2	2	+	+	22-25	7.5-10	zatoriensis
1985	Reymond & Hag.	Morphology	umbrinus	0	0	+	*	*	*	umbrinus
1986	Schlösser	Culture	umbrinus	0	0	+	*	*	*	umbrinus
1987	Korshikov	Systematic	umbrinus/ina	3	0	+	+	25-50	8.5-12.5	umbrinus
1988	Bourrelly	Systematic	minor	0	0	+	*	*	*	NC
1988	Bourrelly	Systematic	minor	0	0	+	*	*	*	NC
1988	Bourrelly	Systematic	zatoriensis	0	0	+	*	*	*	NC
1988	Bourrelly	Systematic	konarekii	0	0	+	*	*	*	NC
1989	Dillard	Floristic	umbrinus	1	0	-	+	27-50	6-12.5	uncertain
1990	Reymond & Hag.	Morphol. system.	umbrinus	27	27	+	-	max. 25	7-10	umbrinus
i.p.	Reymond & Yara.	Systematic	umbrinus	6	5	+	-	19-32	7.5-13	umbrinus
i.p.	Reymond & Duart	Systematic	zatoriensis	9	9	-	+	14-26	6-12.5	zatoriensis
pub.	Reymond & al.	Morphol. System.	umbrinus	16	15	+	-	aver. 25.3	aver. 9.1	umbrinus
sub.	Reymond & al.	Morphol. System.	zatoriensis	7	7	-	+	aver. 19.3	aver. 8.8	zatoriensis

Table 1. Chronological list of authors, reports and data related to *Pachycladella*.

**Explanations of signs:** +: characteristic is present; -: characteristic is absent; \*: no information about the characteristic; **Abbreviations:** NF: number of figures; NOF: number of original figures; CRU: cell described with cruciate appendages; TEF: cell described with tetrahedral appendages; VR: very rarely; SO: sometimes; APPEND. L.: length of the appendages (in  $\mu\text{m}$ ); CELL DIAM.: diameter of the cell (in  $\mu\text{m}$ ); aver.: average; max.: maximum; NC: no comment; i.p.: in press; sub.: submitted.

TAOYUAN			TAOYUAN (cont.)			LA NOUE		
#	App. L.	Cell. D.	#	App. L.	Cell. D.	#	App. L.	Cell. D.
1	18.9	11.0	57	28.0	10.5	1	13.8	8.0
2	19.0	8.3	58	28.0	7.9	2	14.5	7.3
3	19.3	7.9	59	28.0	8.8	3	14.5	6.5
4	19.4	8.8	60	28.0	9.6	4	15.3	10.2
5	20.0	7.9	61	28.0	7.9	5	16.0	8.0
6	20.0	6.6	62	28.0	8.8	6	16.0	8.0
7	21.0	7.9	63	28.2	9.4	7	16.7	7.3
8	21.0	10.5	64	28.5	9.6	8	16.7	8.0
9	21.0	7.0	65	28.9	8.9	9	16.7	8.0
10	21.0	8.8	66	29.4	8.5	10	17.5	6.5
11	21.0	9.6	67	29.4	10.3	11	17.5	8.4
12	21.0	9.6	68	29.8	8.8	12	17.5	9.5
13	21.0	7.7	69	30.6	8.9	13	17.5	6.5
14	21.8	9.1	70	31.2	11.2	14	17.5	7.3
15	21.8	8.2	71	31.5	9.4	15	17.5	8.7
16	21.9	9.6	72	31.8	10.6	16	17.5	9.5
17	21.9	8.8	73	32.4	10.0	17	17.5	11.6
18	21.9	8.8	74	32.4	10.6	18	17.5	6.5
19	22.2	9.4	75	33.3	10.5	19	18.2	7.3
20	22.4	9.1	76	33.3	10.5	20	18.2	6.2
21	22.4	9.4	77	38.8	10.6	21	18.2	10.9
22	22.4	9.7				22	18.2	8.0
23	22.7	10.5				23	18.2	8.0
24	22.8	8.8				24	18.2	11.6
25	22.8	9.6				25	18.2	11.6
26	22.8	7.8				26	18.9	8.0
27	22.6	8.5				27	18.9	12.4
28	22.9	10.0	HAUT RANCON			28	18.9	7.3
29	23.3	8.9	#	App. L.	Cell. D.	29	18.9	8.0
30	23.5	7.6	1	18.9	8.0	30	18.9	8.0
31	23.5	9.1	2	18.9	8.0	31	18.9	9.5
32	24.1	7.1	3	18.9	8.7	32	19.6	8.7
33	24.1	8.8	4	20.4	8.0	33	19.6	10.2
34	24.4	8.9				34	19.6	11.6
35	24.5	8.8				35	19.6	8.0
36	24.5	7.9				36	20.4	10.2
37	24.5	9.6				37	20.4	9.5
38	24.7	8.8				38	20.4	8.7
39	25.0	9.3				39	20.4	8.0
40	25.0	8.8	LA VELLE			40	21.1	8.4
41	25.3	9.4	#	App. L.	Cell. D.	41	21.1	8.0
42	25.4	8.8	1	12.9	7.9	42	21.1	8.0
43	25.7	7.4	2	13.6	7.9	43	21.1	6.7
44	25.9	8.2	3	15.0	8.6	44	21.1	7.3
45	25.9	11.2	4	15.7	7.9	45	21.1	6.5
46	25.9	7.9	5	15.7	9.3	46	21.1	10.2
47	26.3	8.8	6	16.4	8.6	47	21.8	11.6
48	26.3	7.9	7	16.4	6.4	48	22.5	12.4
49	26.3	7.9	8	17.0	7.1	49	22.5	11.6
50	26.3	9.6	9	17.0	7.1	50	23.3	12.4
51	26.4	12.1	10	17.1	8.6	51	23.3	8.7
52	27.1	8.8	11	17.1	7.1	52	23.3	7.3
53	27.1	8.8	12	17.1	8.6	53	24.0	7.6
54	27.1	8.8	13	18.6	9.3	54	24.7	8.7
55	27.2	9.4	14	18.6	10.0	55	25.5	8.7
56	28.0	10.5	15	20.7	10.0	56	26.2	9.5

Table II. Length of appendages (App.L.) and cell diameter (Cell.D.) of all the observed cells of *Pachycladella* (in  $\mu\text{m}$ ).



The samples of Taiwan and La Noue were also employed for a study using a TEM by Raymond & al. (submitted).

## RESULTS

### The pond at Taoyuan (Taiwan).

77 cells have been observed, all with a cruciate disposition of the tubular and bifurcated appendages. The bases and the apexes of the processes form all together a rough plane. The cell body is spherical to slightly quadrangular and posses one central pyrenoid (Figs 4-7 and 16-21). The colour of the appendages is translucent to light yellow. The average length of appendages and cell diameter is 25.3  $\mu\text{m}$  and 9.1  $\mu\text{m}$  respectively. More information is given in Tables II and III. Despite the difference in the length of the appendages when compared to the original description by G.M. Smith (1924) (Fig. 2 and Table I), they have all been determined as *P. umbrina*.

	Taoyuan	Haut Rançon	La Velle	La Noue
Cells nb.	77	4	15	56
Max. L. $\mu\text{m}$	38.8	20.4	20.7	26.2
Min. L. $\mu\text{m}$	18.9	18.9	12.9	13.8
L. aver $\mu\text{m}$	25.31	19.27	16.59	19.33
L. SD	3.96	0.73	1.95	2.74
Max. D. $\mu\text{m}$	12.1	8.7	10.0	12.4
Min. D. $\mu\text{m}$	6.6	8.0	6.4	6.2
D. aver $\mu\text{m}$	9.08	8.18	8.26	8.80
D. SD	1.07	0.42	1.07	1.70
L/D aver	2.81	2.36	2.02	2.26
L/D SD	0.42	0.15	0.29	0.45
Posit. append.	cruciate	tetrahedral	tetrahedral	tetrahedral
Deter. species	<i>P. umbrina</i>	<i>P. zatoriensis</i>	<i>P. zatoriensis</i>	<i>P. zatoriensis</i>

Table III. Basic results obtained from the present records of *Pachycladella*. **Abbreviations:** Cell nb: number of cells recorded per locality; L: length of the appendages; D: diameter of the cell; SD: standard deviation; aver.: average; Posit. appends: Position of the appendages; Deter. species: determination of the species.

### The French reservoirs.

Le Haut Rançon: 4 cells were observed, all with a regular tetrahedral disposition of the appendages. The average length of appendages and cell diameter is 19.3  $\mu\text{m}$  and 8.2  $\mu\text{m}$  respectively. More information is given in Tables II and III.

La Velle: 15 cells were observed, all with a tetrahedral disposition of the appendages. The average length of appendages and cell diameter is 16.6  $\mu\text{m}$  and 8.3  $\mu\text{m}$  respectively. More information is given in Tables II and III.

La Noue: 56 cells were observed, all with a tetrahedral disposition of the appendages (Figs 8 - 15 and 22 - 27). The average length of appendages and



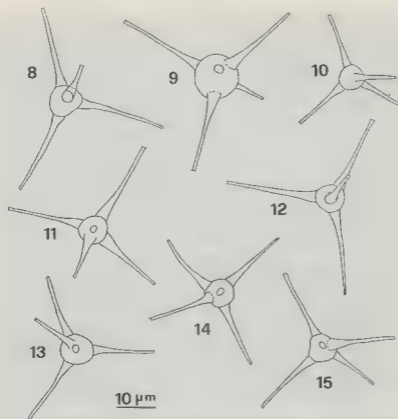


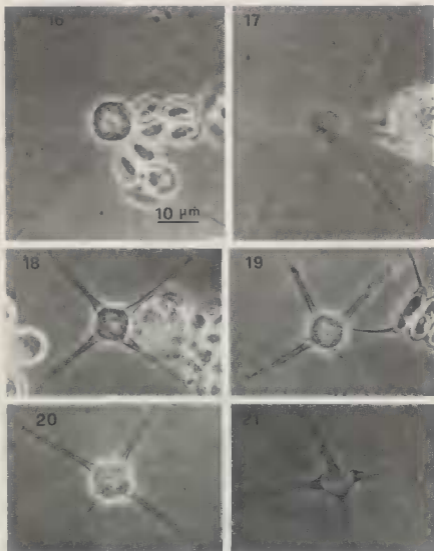
Fig. 8-15. *Pachycladella zatoriensis* from France.

cell diameter is  $19.3 \mu\text{m}$  and  $8.8 \mu\text{m}$  respectively. More information is given in Tables II and III.

All the cells observed in the French reservoirs have a central pyrenoid. The cell body is spherical, polyhedral or quadrilobate. The appendages are more conical and thinner at their bifurcated apex than those of the Taiwan specimens (see above), and their colour is darker. All these cells have been determined as *P. zatoriensis* (Figs 8-15 and 22-27).

For further comparisons of specimens from Taiwan and France with the TEM, refer to the paper of Reymond et al. (submitted).

As additional information, Prof. G.E. Dillard sampled *P. umbrina* in 1990 in Koa Lake, Kentucky, USA (personal communication). The specimen that we could observe on a micrograph has four cruciate and tubular appendages about  $37 \mu\text{m}$  long and a spherical cell body about  $9 \mu\text{m}$  in diameter. It fits the dimensions and the morphology given by G.M. Smith (1924) for *P. umbrina*.

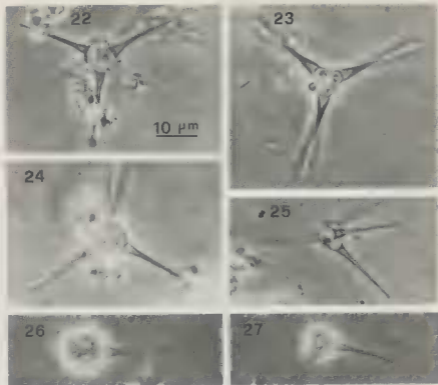


Figs. 16-21. *Pachycladella umbrina* from Taiwan.

## DISCUSSION

### Possible sources of confusion.

The compilation of previous records of *Pachycladella* has shown the confusion which exists at the levels of the generic and specific description. This problem has been enhanced by the polymorphism of the recorded cells.



Figs. 22-27. *Pachycladella zatoriensis* from France.

This confusion can be partly explained by the following comments:

1) G.M. Smith (1924) speaks of the quadrate disposition of the appendages, when his drawings seem to show partly the contrary (Fig. 2A). This certainly allowed, unintentionally, a free interpretation of *P. umbrina*; experience shows that the figures have generally more impact than the description.

2) Depending on the position of the observed cell, the quadrate or the tetrahedral position of its appendages can be inadequately observed and interpreted. For example, despite their cruciate disposition, the apexes of the appendages of *P. umbrina* are not always viewed at the same focus; consequently it could be interpreted as a tetrahedral disposition (Fig. 4).

#### Taxonomy

The present records show clearly that this genus can be divided into two main categories: cells with a cruciate disposition of the appendages which we call *P. umbrina*, and cells with a tetrahedral disposition called *P. zatoriensis*. It also shows that the length of appendages, the cell shape and diameter can notably vary among specimens of the same collection. However all the cells of the four collections have a common feature: four bifurcated appendages and a cen-

tral pyrenoid (confirmed with the TEM by Raymond *et al.* submitted). Without any doubt they belong to the same genus.

In the category of cruciate cells, the length of appendages varies from 18.9  $\mu\text{m}$  (Taiwan) to 120  $\mu\text{m}$  (*P. umbrina* v. *gobindsagarensis*, Jha & Kaushal (1983), India). This is an indication that the category could be divided into several taxonomic categories at the level of the sub-species or even lower. The few data at our disposal (18.9 to 38.8  $\mu\text{m}$  from our work - 35 to 50  $\mu\text{m}$  from Smith (1924) and 96 to 120 from Jha & Kaushal (1983)) presently prevent us from new serious nomenclatural propositions.

In most of the genera or species descriptions carried out, the approximate number of observed cell as well as the average of their measurements has been omitted. Despite the fact that these indications are not always useful at the moment, we think that they could be essential for further taxonomical studies. In the present report Table II serve this role.

#### Descriptions of *Pachycladella* and *P. umbrina*

We have shown that the "quadrate disposition" of *Pachycladella* is a specific characteristic and not at all a generic one. Moreover TEM reports (Reymond & Hegewald, 1990; Reymond *et al.*, submitted) give new information on this algal genus.

Consequently, we propose some slight changes in the diagnosis of the genus and the type species given by G.M. Smith (1924):

Genus *Pachycladella* (G.M. Smith) Silva.

Cells are solitary, spherical or quadrangular to lobated. The cell wall bears four or more appendages. Appendages, when they are four, are quadrate-ly or tetrahedrally placed. The appendages are hollow, translucent to dark brown in colour, stout, gradually tapering to a blunt point that is usually bifurcate. Chloroplast is single, cup-shaped, parietal, with one pyrenoid, and nearly filling the cavity. Reproduction: zoospore can be observed, but more information is needed.

Species *Pachycladella umbrina* (G.M. Smith) Silva.

Characteristics as above. The cell wall bears four appendages in a quadrate disposition (exceptionally with a tetrahedral disposition). The diameter of cell without processes is 7 - 12.5  $\mu\text{m}$ , and the length of processes is 18.9 - 120  $\mu\text{m}$ . Reproduction occurs mainly by zoospores.

#### Short comments about all the taxa of *Pachycladella*.

The genus *Pachycladella* (G.M. Smith) Silva (1970) is actually composed of five taxa: *P. minor*, *P. komarekii*, *P. zatoriensis* and *P. umbrina* and its variety *gobindsagarensis*.

As we know, *P. minor* (D. & H. Chudyba) Silva (1970) has been observed in Poland by its authors only. Unfortunately this material is no longer available (D. Chudyba, personal communication). Its place in the genus *Pachycladella* is still questionable without additional data. For Fott & Kováčik (1975) this organism has probably another taxonomical position. Komárek & Fott (1983) make the hypothesis that it could be the spore of another algal species.

*P. komarekii* (Fott & Kováčik) Reymond (1980) has been observed in Czechoslovakia by its authors and perhaps a few algologists. Unfortunately this

material is not available (Komárek and Kováček, personal communications). This species is possibly a *Pachycladella*, which in this case would seem close to *P. zatoriensis*, because of its tetrahedrally arranged appendages. However it needs further investigations.

*P. zatoriensis* (Bednarz et Mrozinska-Webb) Komarek (1979) is characterized by a tetrahedral disposition of four appendages with a bifurcate apex. More details are given in the present report and by Reymond *et al.* (submitted).

*P. umbrina* (G.M. Smith) Silva (1970), the type species is characterized by a cruciate disposition of four appendages with a bifurcate apex. More details are given in the present report and by Reymond *et al.* (submitted).

*P. umbrina* v. *gobindsagarensis* was recently proposed by Jha & Kaushal (1983). Unfortunately it was not possible to obtain any material from the authors. By the huge size of its cruciate appendages, this taxon represents the biggest cells of *Pachycladella*.

#### ACKNOWLEDGEMENTS

We are indebted to Prof. G.E. Dillard of Kentucky (USA), Dr. E. Hegewald of Jülich (Germany), Dr. D. Chudyba of Olsztyn-Kortowo (Poland), Dr. T. Bednarz of Cracow (Poland), Dr. J. Komárek and Dr. L. Kováček of Třeboň for their personal information concerning *Pachycladella*. We thank also Dr. J.-P. Hornung and Dr. M. von Schack of Lausanne (Switzerland) and Dr. J.-P. Dubois of Thonon (France) for critically reading the manuscript.

The first author is also affiliated and indebted to the Laboratoire de Microbiologie générale of the Département de Biologie végétale of the University of Geneva and to the Musée et Jardin botanique de Lausanne, Switzerland.

#### BIBLIOGRAPHY

- BARTA Z., FELFÖLDY L., HAJDU L., HORVÁTH K., KISS K., SCHMIDT A., TAMAS G., UHERKOVICH G. & VÖRÖS L., 1976 - A zöldalgák (Chlorococcales) rendjének kishatározója. In FELFÖLDY L. (Ed.), *Vizügyi Hidrobiológia*. Vol. 4, Budapest, 343 p.
- BEDNARZ T. & MROZINSKA-WEBB T., 1971 - A new species of the genus *Pachycladon* G.M. Smith found on the terrain of Zator. - Nowy gatunek z rodzaju *Pachycladon* G.M. Smith znalezione na terenie Zatora. *Fragm. Flor. Geobot.* 17: 171-173.
- BEDNARZ T. & NOWAK M., 1972 - Wybrane gatunki glonów, znalezione w stawach karpowych kompleksu Łaskowa koło Zatora - Selected species of algae found in carp ponds of the Łaskowa complex near Zator. *Acta Hydrobiol. (Krakow)* 14: 103-112.
- BERNARD CH., 1908 - *Protococcales et Desmidiées d'eau douce, récoltées à Java*. Dép. Agric. aux Indes Néerlandaises, Batavia, 230 p.
- BOURRELLY P., 1966 - *Les algues d'eau douce. Initiation à la systématique. I. Les algues vertes*. Boubée N. & Cie. Paris, 511 p.
- BOURRELLY P., 1972 - *Les algues d'eau douce. Initiation à la systématique. I. Les algues vertes*. Réimpression revue et augmentée. Boubée N. & Cie., Paris, 572 p.
- BOURRELLY P., 1988 - *Complément. Les algues d'eau douce. Initiation à la systématique. I. Les algues vertes*. Soc. nouvelle édit. Boubée, Paris, 182 p.
- CAPDEVILLE P., 1985 - Observations dans la région des Landes d'algues d'eau douce rares ou nouvelles pour la flore de France. *Cryptogamie, Algol.* 6: 141-170.

- CHUDYBOWA D. & CHUDYBA H., 1965 - *Raphidionema longiseta* Vischer. i *Pachycladon minus* n. sp. w zielonym smiegu z okolic Olsztyna. *Acta Hydrobiol.* 7: 297-302.
- DILLARD G.E. 1989 - *Freshwater Algae of the Southeastern United States. Part 1. Chlorophyceae, Volvocales, Tetrasporales and Chlorococcales.* Cramer J., Stuttgart. *Bibliotheca Phycologica* 81. 202 p.
- DILLARD G.E., MOORE S. & GARRETT L. 1976 - Kentucky algae, II. *Trans. Kentucky Acad. Sci.* 37: 20-25.
- FOTT B., 1960 - Zur Kenntniss der Gattung *Saturnella* (Chlorococcales). *Nova Hedwigia* 2: 273 - 278.
- FOTT B. & KOVÁČIK L., 1975 - Über die Gattung *Treubaria* (Chlorococcales, Chlorophyceae). *Preslia* 47: 305-316.
- GARCIA DE EMILIANI M.O., MOSTO P. & TELL G. 1976 - Algas de agua dulce nuevas o poco conocidas en la Argentina. *Physis Sec. B.* 35: 185-198.
- HARMON ENGINEERING AND TESTING COMPANY, INC., 1983 - *Water quality management studies. Middle Black Warrior and Lower Tombigbee Rivers, Warriors and Demopolis Lakes, July 1978 - December 1979.* Report prepared for U.S. Army Corps of Engineers Environmental Quality section. P.O. Box 2288. Mobile, Alabama 36628. 500 p.
- JHA B.C. & KAUSHAL D.K., 1983 - Algae from Gobindasagar, Himachal Pradesh. *Phykos* 22: 141-143.
- KOMÁREK J. 1979 - Änderungen in der Taxonomie der Chlorokokkalalgen. *Arch. Hydrobiol. Suppl.* 56. *Algological Studies* 24: 239-263.
- KOMÁREK J. & FOTT B. 1983 - Chlorophyceae (Grünalgen), Ordnung: Chlorococcales. In Huber-Pestalozzi G., *Das Phytoplankton des Süßwassers.* Die Binnengewässers 16, 7'1: 1044 p.
- KORSIIKOV O.A., 1953 - *Pidklas Protokokovi (Protococcineae) Vakuol'ni (Vacuolales ta Protokokovi (Protococcales).* *Viznaentik prsnovodnih vodorostejukrains'koi RSR.* 5. Vidav. Akad. Nauk R.S.R., Kiev, 449 p.
- KORSIIKOV O.A., 1987 - *The Freshwater algae of the Ukrainian SSR. V. Sub-Class Protococcineae. Vacuolales and Protococcales.* Translated by J.W.G. LUND and W. TYLKA. BISHEN SINGH MAHENDRA PAL SINGH and KOELTZ Scientific Books (Eds). 412 p.
- LACKEY J., 1958 - The suspended microbiota of the Clinch River and adjacent waters in relation to radioactivity in the summer of 1956. *Engr. Progress, Univ. Florida* 12: 1-26.
- McINTYRE B.B., 1939 - A check list of the algae of Kentucky. *Castanea* 4: 27-37.
- METEE M.F., HARRIS S.C. & MALATINO A.M., 1984 - *Water quality management studies. Postimpoundment study of R.E. "Bob" Woodruff Lake, Alabama River, Alabama, August-December 1977 and April-December 1978.* Report prepared for the U.S. Army corps of Engineers Environmental Quality Section. P.O. Box 2288. Mobile, Alabama 36628. 520 p.
- PHILIPPOSE M.T., 1967 - *Chlorococcales.* I.C.A.R. New Dehli. 365 p.
- PLAYFAIR G.I., 1918 - New and rare freshwater algae. *Proc. Lin. Soc. New South Wales* 43: 497-543.
- PRESCOTT G.W., 1964 - *How to know the fresh-water algae.* JAUQUES H.E. (Ed.). Pictured key nature serie. Dubuque, Iowa, W.M.C. Brown Comp. Publisher. 272 p.
- PRINTZ H., 1927 - Chlorophyceae. In ENGLER A., *Die natürlichen Pflanzenfamilien* 3. ENGELMANN W. (Ed.), Leipzig, 463 p.
- REYMOND O.L., 1980 - Contribution à l'étude de *Treubaria* Bernard (Chlorococcales, Chlorophyceae). *Candollea* 35: 37-70.

- REYMOND O.L. & KOUWETS F.A.C., 1984 - Taxonomical and Ultrastructural survey of the Genus *Desmatriactum* West & West (Chlorococcales). In IRVINE D.E.G. & JOHN D.M.(Eds), *Systematics of the Green Algae*. Systematics associations Special Vol. 27, London and Orlando, Academic Press, pp. 379-389.
- REYMOND O.L. & HEGEWALD E., 1985 - *Pachycladella umbrina* (G.M. Smith) Silva (Chlorococcales): A morphological and life cycle study. Book of abstracts, 2nd International Phycological Congress. University of Copenhagen. p. 63.
- REYMOND O.L. & HEGEWALD E., 1990 - Morphology, life cycle and taxonomy in *Pachycladella umbrina* (Chlorophyceae, Chlorococcales). *Arch. Hydrobiol. Suppl.* 85. *Algalogical studies* 58: 15-28.
- REYMOND O.L. & DRUART J.C. In press - *Pachycladella zatoriensis*. In YAMAGISHI T. & AKIYAMA (Eds.), *Photomicrographs of the Fresh-water Algae*. Tokio, Uchida Rokakuho.
- REYMOND O.L. & YAMAGISHI T. In press - *Pachycladella umbrina*. In YAMAGISHI T. & AKIYAMA (Eds.), *Photomicrographs of the Fresh-water Algae*. Tokio, Uchida Rokakuho.
- REYMOND O.L., BIRCHEM R. & BOUTINARD ROUELLE-ROSSIER V., Submitted - Cell morphology and localization of Fe and Mn in field-collected algae *Pachycladella umbrina* and *P. zatoriensis* (Chlorococcales, Chlorophyceae). *Algal. Studies*.
- SCHLÖSSER I.W., 1986 - Sammlung von Algenkulturen Göttingen: Additions to the Collection since 1984. *Ber. Deutsch. Bot. Ges.* 99: 161-168.
- SILVA P.C., 1970 - Remarks on algal nomenclature IV. *Taxon* 19: 941 - 945.
- SMITH G.M., 1924 - Ecology of the plankton algae in Palisades Interstate Park, including the relation of control methods to fish culture. *Roosevelt Wild Life Bulletin* 2: 95-195.
- SMITH G.M., 1933 - *The fresh-water algae of the United States*. MacGraw-Hill, New York. 716 p.
- SMITH G.M., 1950 - *The fresh-water algae of the United States*. 2nd edit. MacGraw-Hill, New York. 719 p.
- WAWRIK F., 1977 - Beobachtungen an *Pachycladon umbrinus*, *Micractinium bornhemense*, *Paradoxia multiseti* und der Infektion einer *Mallomonas*-Population. *Arch. Protistenk.* 119: 60-67.
- WHITFORD L.A., 1936 - New and little known algae from North Carolina. *J. Elisha Mitchell Sci. Soc.* 52: 93-98.
- WHITFORD L.A., 1943 - The fresh-water algae of North Carolina. *J. Elisha Mitchell Sci. Soc.* 59: 131 - 170.
- WHITFORD L.A., 1958 - Phytoplankton in North Carolina lakes and ponds. *J. Elisha Mitchell Sci. Soc.* 74: 143 - 157.
- WHITFORD L.A. & SCHUMACHER G.J., 1973 - *A manual of fresh-water algae*. Raleigh, N.C., Sparks Press, 323 p.